In the experimental part of this disclosure the procedures used for purification and characterization of the present novel APC-cofactor 2 activity is described and its connection with Factor V is verified.

In summary, the evidences for the presence of the APC-cofactor 2 activity on Factor V are:

1. The procedure designed for the isolation of APC-cofactor 2 activity and earlier methods for isolation of Factor V are very similar. On SDS-PAGE three bands appear at approximately 200-220 kDa (C-terminal portion), 140-160 kDa (N-terminal portion) and 330 kDa which also is very similar to what has been reported for Factor V. (Cf the experimental section of the disclosure and Dahlbäck et al, J. Clin. Invest. 66 (1980) 583-91.) The intensity of the band at 330 kDa is enhanced for both APC-cofactor 2 activity and Factor V when higher concentrations of protease inhibitors are used during the purification procedure. For instance, a benzamidine hydrochloride concentration of 10 mM gives rise to a significant band at 330 kDa.

2. Specific polyclonal antiserum against human Factor V (Dakopatt A/S, Denmark) reacts with each of the three bands associated with APC-cofactor 2 activity in Western blotting.

3. After addition of thrombin to the present preparations comprising APC-cofactor 2 activity the three bands disappear and the products obtained become indistinguishable from the products formed by thrombin activation of Factor V.

4. Seventeen monoclonal antibodies reacting with Factor V have been obtained by using a preparation purified in respect of APC-cofactor 2 activity as immunogen. Two of the monoclonal antibodies partially inhibited APC-cofactor 2 activity without inhibiting Factor V procoagulant activity.

5. Factor V procoagulant activity and APC-cofactor 2 activity are coeluted on every chromatographic material tested, Heparin Sepharose, Blue-Sepharose, Wheat Germ Lectin Sepharose, Q-Sepharose and S-Sepharose (Pharmacia, Sweden) illustrating materials that have been tested.

6. Both Factor V procoagulant activity and APC-cofactor 2 activity are retained on a matrix carrying polyclonal antibodies against human Factor V (Dakopatts A/S,

Denmark).

7. Both Factor V procoagulant activity and APC-cofactor 2 activity are retained on matrices, such as Sepharose and Affigel, carrying antisera against different fragments of bovine Factor V, which cross-react with human Factor V.

8. Both Factor V procoagulant activity and APC-cofactor 2 activity are retained and coeluted on a chromatographic support, such as Affigel, carrying a high affinity monoclonal antibody, which had been prepared by using a preparation purified in respect of APC-cofactor 2 activity as immunogen. In itself, this antibody inhibited neither APC-cofactor 2 activity nor Factor V procoagulant activity. Elution was performed at a pH of approximately 10.5-11.

9. A recent publication disclosing that autoantibodies against Factor V may result in thrombosis (Kapur A et al, A.J. Hematol. 42 (1993) 384-388).

Preparations enriched in APC-cofactor 2 activity have been obtained by the same methods as have been used previously for the isolation of Factor V. It has been found that divalent metal ions, such as calcium ion, have a stabilizing effect on the APC-cofactor 2 activity and, hence, calcium ions were added during the purification.

Essentially the same purification procedure has been used as a first attempt in order to elucidate the novel activity disclosed in the above mentioned WO 93/10261. According to the results presented herein, the novel activity has been identified as a cofactor activity to APC expressed as a novel property of Factor V, or, possibly, a complex or fragments thereof as discussed above. Thus, alternative and simpler preparation methods will become available. Current methods, such as gel chromatography, affinity chromatography with e.g. anti-APC-cofactor 2 activity antibody as affinity ligand, ion exchange chromatography, etc, have been used, suitably after improvement. In addition, methods based on DNA-recombinant technique may be applicable.

Accordingly, the present invention is also related to a preparation derived from blood or blood related products, such as plasma, said preparation being purified in respect of a blood coagulation component, which can express anticoagulant activity as a cofactor to APC thereby enhancing its proteolytic